

Original Article

## PTH and 1.25 vitamin D response to a low-calcium diet is associated with bone mineral density in renal stone formers

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### Abstract

**Background.** Renal calcium stones and hypercalciuria are associated with a reduced bone mineral density (BMD). Therefore, the effect of changes in calcium homeostasis is of interest for both stones and bones. We hypothesized that the response of calciuria, parathyroid hormone (PTH) and 1.25 vitamin D to changes in dietary calcium might be related to BMD.

**Methods.** A single-centre prospective interventional study of 94 hyper- and non-hypercalciuric calcium stone formers consecutively retrieved from our stone clinic. The patients were investigated on a free-choice diet, a low-calcium diet, while fasting and after an oral calcium load. Patient groups were defined according to lumbar BMD (z-score) obtained by dual X-ray absorptiometry (group 1: z-score  $< -0.5$ ,  $n = 30$ ; group 2: z-score  $-0.5$ – $-0.5$ ,  $n = 36$ ; group 3: z-score  $> 0.5$ ,  $n = 28$ ). The effect of the dietary interventions on calciuria, 1.25 vitamin D and PTH in relation to BMD was measured.

**Results.** An inverse relationship between BMD and calciuria was observed on all four calcium intakes ( $P = 0.009$ ). On a free-choice diet, 1.25 vitamin D and PTH levels were identical in the three patient groups. However, the relative responses of 1.25 vitamin D and PTH to the low-calcium diet were opposite in the three groups with the highest increase of 1.25 vitamin D in group 1 and the lowest in group 3, whereas PTH increase was most pronounced in group 3 and least in group 1.

**Conclusion.** Calcium stone formers with a low lumbar BMD exhibit a blunted response of PTH release and an apparently overshooting production of 1.25 vitamin D following a low-calcium diet.

**Keywords:** 1.25 vitamin D; bone mineral density; hypercalciuria; nephrolithiasis; parathyroid hormone

### Introduction

In the western world, renal calcium stone disease has a life-time prevalence of about 10% [1]. Over the last 30 years, epidemiological evidence associating renal calcium stone disease and decreased bone mineral density (BMD) has accumulated [2–6]. By using single photon absorptiometry [6,7], quantitative computed tomography [2,8], dual photon absorptiometry [3] and dual energy X-ray absorptiometry [4,5], a diminished BMD was found at the lumbar spine [2,3,8], radius [4,6,7] and tibia [5] in calcium stone formers. The clinical relevance of this association was underlined by large retrospective cohort studies showing an increased risk of vertebral fractures in calcium stone formers [9,10]. Furthermore, the decreased BMD in calcium stone formers was linked to hypercalciuria in most [2–4,7,8], but not all [5], studies. Calciuria is a continuous trait and is the final result of numerous regulatory processes, including intestinal calcium absorption, bone resorption and renal reabsorption. Defining hypercalciuria is difficult and attempts by Pak *et al.* are often used as a reference [11–13]. These protocols allow us to subclassify hypercalciuria according to calcium excretion during the intake of different amounts of calcium. In short, idiopathic hypercalciuria is defined either as fasting (also called dietary independent and including renal calcium leak) when occurring under a low-calcium diet, or as absorptive (also called dietary dependent) when occurring after an oral calcium load. Using this definition, fasting [3,8] as well as absorptive hypercalciuria [2,4] has been found to be associated with a diminished BMD in some studies. Higher 1.25 vitamin D plasma concentrations and lower PTH levels were encountered under free-choice diet conditions in patients diagnosed with absorptive and fasting hypercalciuria when compared to normocalciuric stone formers [2,4,8,14]. In line with these reports, an elevated production rate of 1.25 vitamin D has been reported in absorptive hypercalciuria [15,16].

In contrast to the numerous reports on hypercalciuria and its association with diminished BMD, reports that study the BMD and the urinary calcium excretion of stone formers with regard to the regulation of mineral metabolism under different calcium intakes are rare. Bataille *et al.* [2] found a positive correlation between 1.25 vitamin D and BMD

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when measured in a subgroup of 24 calcium stone formers with dietary-independent hypercalciuria under fasting conditions. However, no data on PTH levels were reported in this study.

We hypothesize now that PTH and 1.25 vitamin D change differently when calcium stone formers with high, intermediate and low BMD are challenged for diagnostic purposes by a low-calcium diet for 1 week. As the reference site for the analysis of BMD we chose the lumbar spine that is the only skeletal site exhibiting a higher fracture rate in stone formers when compared to non-stone formers [9,10]. The present investigation revealed that when all calcium stone formers were considered as one group, a low-calcium diet decreased calcaemia and calciuria, and increased PTH and 1.25 vitamin D levels. However, when the patients were grouped according to their BMD, a differential BMD-dependent response of PTH and 1.25 vitamin D to the low calcium diet was observed.

## Methods

### Patient data

All patients were seen for an outpatient metabolic work-up at our renal stone clinic between March 2004 and June 2006 and were included into the study independently of their degree of calciuria. Inclusion criteria were as follows: (i)  $\geq 18$  years of age of either gender and (ii) passage of at least one calcium-containing kidney stone, defined either by stone analysis or by the presence of opaque material on conventional radiograph or computed tomography in the absence of cystinuria. Exclusion criteria were (i) established cause of calcium stone formation, such as primary hyperparathyroidism, overt distal renal tubular acidosis, sarcoidosis, excessive vitamin D intake, hypercalciuria due to hypercalcaemia (immobilization or malignancy) and primary or enteric hyperoxaluria (exclusion of patients with inflammatory bowel disease and short bowel syndrome); (ii) creatinine clearance  $> 60$  ml/min as calculated from the serum and urinary creatinine of two 24-h urine collections and (iii) urinary tract infection. Patients were asked to stop calcium supplements or any drug that could affect the metabolism of calcium during the evaluation. All patients gave their consent for their participation and the protocol was approved by the institutional review board of the University Hospital Bern.

Ninety-four patients met the above-mentioned criteria. The results of the first 30 patients recruited had been pre-

sented at the 38th American Society of Nephrology Annual Renal Week Meeting 2005 at Philadelphia (Abstract 554925). The results derived from these 30 patients were similar to those of the 64 subjects recruited thereafter. Therefore, the results of all 94 patients were pooled.

### Ambulatory protocol

All patients underwent a three-visit mineral metabolism work-up including an osteodensitometry. A routine clinical assessment including a physical examination and history was performed. Mineral metabolism was investigated on four different calcium regimens according to a protocol first established by Pak *et al.* [11]: (i) free-choice diet, (ii) low-calcium diet, (iii) fasting and (iv) after a 1 g oral calcium load (Figure 1).

- (i) While on their normal free-choice diet, patients led a 5-day dietary diary [13], collected 24-urines twice, and had a blood sample drawn.
- (ii) The dietary diaries were analysed by a nutritionist for meat and calcium intake. Then patients were instructed to follow a diet restricted in calcium (10 mmol or 400 mg/day) and sodium (100 mmol/day) under ambulatory conditions for 7 days. On the last day, a 24-h urine collection was obtained which partially overlapped with a 12-h overnight fasting period.
- (iii) The next day, the second ambulatory visit took place in our clinic. Blood was drawn and 2-h urine was collected under fasting conditions.
- (iv) After completion of this collection, patients received 1 g of calcium orally (Calcium Sandoz ff<sup>®</sup>, containing calcium carbonate, lactate and gluconate in 300 ml distilled water). After this calcium load, a 4-h urine collection was performed.

In the 24-h urines, volume, sodium, phosphate, calcium, magnesium, creatinine, uric acid and urea were measured by standard laboratory techniques with an automated analyser. Sulfate and oxalate were measured by high performance liquid chromatography (Dionex DX-600 analyser). Deoxypyridinoline was measured by an immunoassay (DPC, Immunolite 2500) in the urine samples obtained during the free-choice diet. In the 2- and 4-h urines, volume, calcium and creatinine were assessed by standard laboratory methods. In the blood samples sodium, phosphate, calcium, uric acid and creatinine were assessed by standard laboratory methods. Intact parathyroid hormone was measured by an enzyme-immunoassay (Roche Modular E170), and 1.25

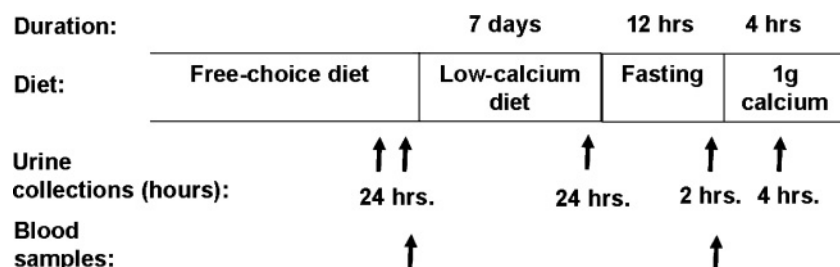


Fig. 1. Schematic illustration of the protocol. Distances are not drawn to scale.

vitamin D was also measured by an enzyme-immunoassay (Immundiagnostik AG). Blood for the measurement of total alkaline phosphatase was taken while the subjects were on the free-choice diet and was measured by standard laboratory methods.

Hypercalciuria was defined as either fasting or absorptive hypercalciuria. Fasting hypercalciuria was defined as an elevated fasting urinary calcium [ $\geq 0.00275$  mmol/dl glomerular filtrate (dl GF) or  $\geq 0.11$ , mg/dl GF] in the 2-h urine sample as described by Levy *et al.* [13]. Absorptive hypercalciuria was defined as a urinary calcium-to-creatinine ratio of  $\geq 0.56$  mmol/mmol ( $\geq 0.20$ , mg/mg) in the 4-h urine sample after the oral intake of 1-g calcium [13]. Osteodensitometry was performed by dual-energy X-ray absorptiometry at the second to fourth lumbar vertebra, femoral neck and tibial dia- and epiphysis (DXA, Hologic® scanner QDR 1000W).

### Statistical analysis

Results are expressed as mean  $\pm$  SEM. Comparisons within groups (between diets) were carried out by Student's *t*-test for paired observations. The chi-square likelihood ratio test was used to compare the number of normo- and hypercalciuric patients within the groups. ANOVA, followed by the Student–Newman–Keuls *t*-test for comparison of means (*post hoc* analysis) was used for comparison between groups. Reported *P*-values (two-tailed) of  $\leq 0.05$  were considered significant.

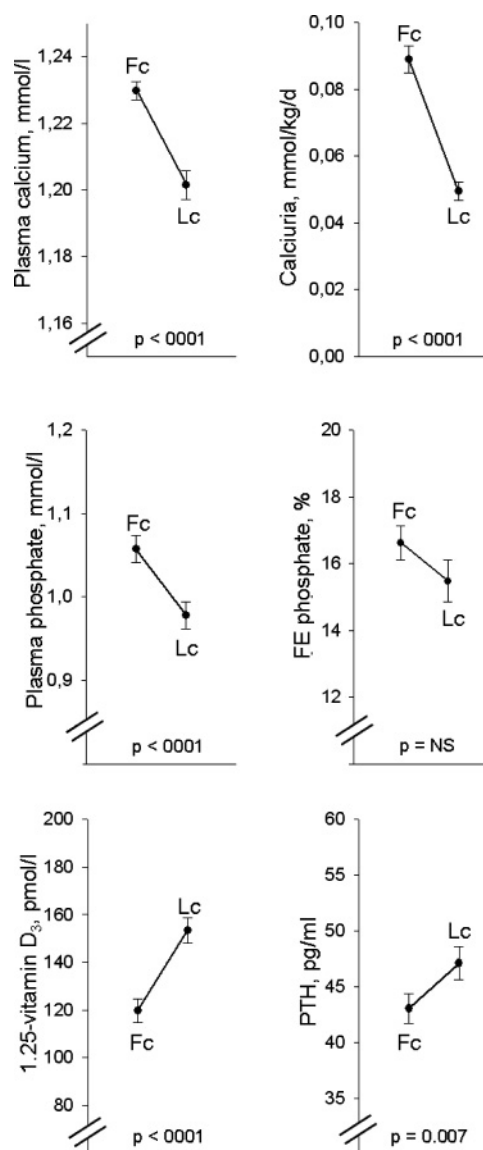
## Results

### Effects of a low-calcium diet

Urine and plasma values of 94 calcium stone-forming patients were investigated. The number of female and male patients in our study (18 versus 76) reflects the gender difference of stone-forming propensity reported in the literature [1]. Analyses were performed on four different dietary calcium intakes (Figure 1) including their usual free-choice diet and after 1 week of a diet low in calcium and sodium content—for simplicity termed 'low-calcium diet' hereafter. Figure 2 shows the impact of these two diets on relevant variables of mineral metabolism. The low-calcium diet caused a significant decline of plasma calcium, plasma phosphate levels, the fractional excretion of phosphate and urinary calcium excretion. Furthermore it caused an increase of the 1.25 vitamin D and PTH concentrations.

### Bone mineral density as a grouping variable

Patients were divided into three subgroups according to their *z*-score at the lumbar spine (group 1: *z*-score  $< -0.5$ ; group 2: *z*-score  $-0.5$ – $0.5$ ; group 3: *z*-score  $> 0.5$ ) (Table 1). There were 30 patients (5 females, 25 males) in group 1, 36 (6 females, 30 males) in group 2 and 28 (7 females, 21 males) in group 3. Of the five postmenopausal women included in this study, four belonged to group 2 and one to group 3. No significant differences



**Fig. 2.** Effect of free-choice (Fc) and low-calcium diet (Lc) on plasma concentrations of calcium, phosphate, 1.25 vitamin D, parathyroid hormone (PTH), calciuria and fractional excretion (FE) of phosphate in all patients investigated ( $n = 94$ ). Error bars indicate SEM.

between groups were detected regarding age, body height, weight, body mass index, family history of stones, stone recurrences and the number of stone events per patient. Upon evaluation of the 5-day dietary diaries, there was a slight but non-significantly higher calcium and meat intake in group 2 when compared to group 1.

Plasma and urine values on free-choice diet and after 1 week of low-calcium diet are given in Tables 2 and 3. On free-choice diet no significant difference between the three groups in any plasma or urine parameter was detected.

Plasma and urinary sodium and calcium, as well as urinary phosphate, uniformly declined in all groups while on a low-calcium diet. Plasma phosphate declined in groups 2 and 3 but not group 1 on the low-calcium diet (Tables 2 and 3). Plasma uric acid levels homogenously increased in all groups (Table 2), without a change in uric acid excretion

**Table 1.** Baseline characteristics of patients grouped according to their *z*-scores at lumbar spine

	All ( <i>n</i> = 94)	Group 1 <i>z</i> -score < -0.5 ( <i>n</i> = 30)	Group 2 <i>z</i> -score -0.5–0.5 ( <i>n</i> = 36)	Group 3 <i>z</i> -score > 0.5 ( <i>n</i> = 28)
Female/male	18/76	5/25	6/30	7/21
Age (years)	46.3 ± 1.3	45.1 ± 2.3	46.6 ± 2.2	47.1 ± 2.1
Body height (cm)	173.6 ± 0.9	173.0 ± 1.3	173.0 ± 1.3	174.5 ± 2.0
Body weight (kg)	77.2 ± 1.6	74.7 ± 2.8	78.8 ± 2.4	78.0 ± 3.1
BMI (kg/m <sup>2</sup> )	25.5 ± 0.4	24.8 ± 0.8	26.2 ± 0.7	25.5 ± 0.8
Osteodensitometry				
<i>z</i> -score lumbar spine	0.04 ± 0.10	-0.92 ± 0.07	-0.08 ± 0.04	1.21 ± 0.13
<i>t</i> -score lumbar spine	-0.50 ± 0.10	-1.40 ± 0.10	-0.66 ± 0.08	0.66 ± 0.14
Normocalciuria	67	16	28	23
Fasting hypercalciuria	9	5	2	2
Absorptive hypercalciuria	18	9	6	3
Stone composition				
Calcium oxalate	44	15	17	12
Calcium phosphate	4	1	2	1
Radio-opaque concrement	46	14	17	15
Stone recurrence, % of patients	86	80	92	86
Number of stone events per patient	2.9 ± 0.1	2.8 ± 0.2	3.3 ± 0.2	2.7 ± 0.2
Family history of stones, % of patients	35	40	33	32
Dietary diary on free-choice diet				
Calcium intake (g/day)	809 ± 42	760 ± 64	906 ± 74	747 ± 76
Meat intake (g/kg/day)	1.69 ± 0.10	1.45 ± 0.14	1.88 ± 0.17	1.72 ± 0.19

**Table 2.** Comparison of plasma parameters on free-choice (Fc) and low-calcium (Lc) diet

	Diet	All patients ( <i>n</i> = 94)	Group 1 <i>z</i> -score < -0.5 ( <i>n</i> = 30)	Group 2 <i>z</i> -score -0.5–0.5 ( <i>n</i> = 36)	Group 3 <i>z</i> -score > 0.5 ( <i>n</i> = 28)	ANOVA group 1 versus 2 versus 3
Creatinine (μmol/l) (45–04)	Free-choice	78 ± 2	75 ± 2	77 ± 3	83 ± 4	0.11
	Low calcium	81 ± 2	78 ± 2	80 ± 2	86 ± 4	0.15
	<i>P</i> (Fc versus Lc)	<0.001	0.02	0.004	0.18	
Sodium (mmol/l) (132–142)	Free-choice	142 ± 0.2	141 ± 0.3	142 ± 0.3	142 ± 0.4	0.19
	Low calcium	139 ± 0.2	139 ± 0.3	139 ± 0.4	140 ± 0.4	0.46
	<i>P</i> (Fc versus Lc)	<0.001	<0.001	<0.001	<0.001	
Calcium (mmol/l) (1.13–1.30)	Free-choice	1.23 ± 0.00	1.23 ± 0.00	1.23 ± 0.01	1.23 ± 0.00	0.57
	Low calcium	1.20 ± 0.00	1.20 ± 0.01	1.21 ± 0.01	1.19 ± 0.01	0.16
	<i>P</i> (Fc versus Lc)	<0.001	<0.001	0.002	<0.001	
Phosphate (mmol/l) (0.74–1.55)	Free-choice	1.06 ± 0.02	1.01 ± 0.03	1.08 ± 0.03	1.09 ± 0.03	0.10
	Low calcium	0.98 ± 0.02	0.97 ± 0.03	0.98 ± 0.03	0.99 ± 0.03	0.80
	<i>P</i> (Fc versus Lc)	<0.001	0.12	0.002	<0.001	
Uric acid (μmol/l) (140–340)	Free-choice	307 ± 9	306 ± 15	309 ± 16	305 ± 16	0.98
	Low calcium	354 ± 11	347 ± 16	364 ± 16	350 ± 25	0.79
	<i>P</i> (Fc versus Lc)	<0.001	<0.001	<0.001	0.05	
PTH (pg/ml) (10–73)	Free-choice	43.0 ± 1.3	43.1 ± 2.2	43.2 ± 2.1	42.8 ± 2.7	0.99
	Low calcium	47.1 ± 1.5	44.7 ± 2.4	46.6 ± 2.3	50.3 ± 3.0	0.31
	<i>P</i> (Fc versus Lc)	0.007	0.55	0.13	0.01	
1.25 vitamin D (pmol/l) (78–160)	Free-choice	120 ± 5.0	127 ± 9.8	113 ± 7.9	121 ± 8.1	0.52
	Low calcium	153 ± 5.3	166 ± 9.8	158 ± 8.2	135 ± 9.3	0.07
	<i>P</i> (Fc versus Lc)	<0.001	<0.001	<0.001	0.13	
Alkaline phosphatase, (U/l) (36–120)	Free-choice	65.0 ± 1.8	65.1 ± 2.9	64.2 ± 2.9	65.8 ± 3.4	0.93

The numbers in parentheses indicate the normal range of values.

(Table 3). Alkaline phosphatase activity was comparable between the three groups (Table 2). Creatinine clearance decreased slightly in all groups, but significance was reached in group 3 only (Table 3). No differences among groups were found for plasma venous pH and bicarbonate, and urinary volume, creatinine, urea, sulfate, oxalate, citrate and deoxypyridinoline/creatinine ratio (data not shown).

In Figure 3 the relationship between 1.25 vitamin D and PTH in the three patient groups is given. On free-choice

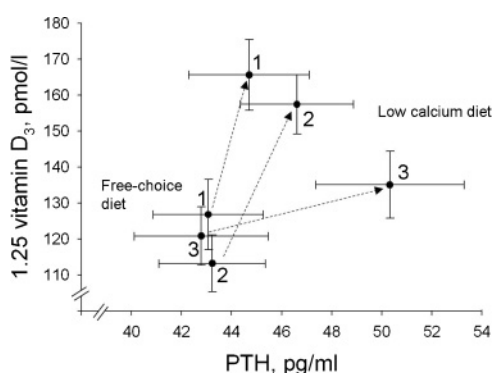
diet, no statistically significant differences between the three groups were found (Table 2). On the low-calcium diet, however, a BMD-dependent disparity was encountered. The 1.25 vitamin D concentration increased mainly in groups 1 and 2, whereas PTH increased mainly in group 3 (Figure 3, ANOVA *P* = 0.03 for the difference of the 1.25 vitamin D-to-PTH ratios on low-calcium diet, *post hoc* analysis *P* < 0.05 for group 1 versus group 3). No gender difference was encountered for this ratio (ANOVA *P* = 0.76).

**Table 3.** Comparison of 24-h urine parameters on free-choice (Fc) and low-calcium (Lc) diet

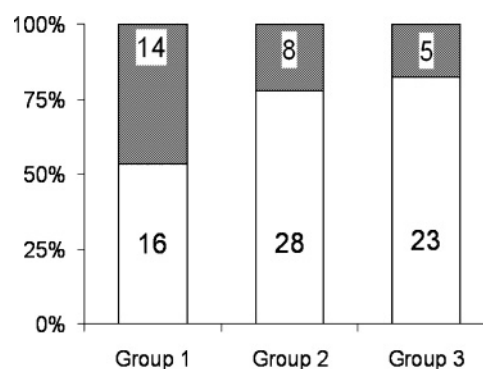
	Diet	All patients (n = 94)	Group 1 z-score <-0.5 (n = 30)	Group 2 z-score -0.5-0.5 (n = 36)	Group 3 z-score >0.5 (n = 28)	ANOVA group 1 versus 2 versus 3
Creatinine clearance (ml/min) (75-142)	Free-choice	123 ± 3	130 ± 6	122 ± 4	117 ± 7	0.31
	Low calcium	117 ± 4	126 ± 8	117 ± 5	107 ± 5	0.13
	P (Fc versus Lc)	0.02	0.48	0.24	0.02	
Sodium (mmol/day) (127-287)	Free-choice	194 ± 7	195 ± 14	190 ± 11	197 ± 11	0.91
	Low calcium	96 ± 5	101 ± 9	93 ± 8	94 ± 6	0.73
	P (Fc versus Lc)	<0.001	<0.001	<0.001	<0.001	
Calcium (mmol/day) (2.5-7.5)	Free-choice	6.85 ± 0.34	7.26 ± 0.66	6.84 ± 0.52	6.40 ± 0.41	0.62
	Low calcium	3.79 ± 0.22	4.17 ± 0.43	3.71 ± 0.33	3.50 ± 0.36	0.45
	P (Fc versus Lc)	<0.001	<0.001	<0.001	<0.001	
Calcium (mmol/2 h)	Fasting	0.23 ± 0.02	0.29 ± 0.04	0.21 ± 0.02	0.20 ± 0.03	0.12
Calcium (mmol/4 h)	1 g oral calcium load	0.97 ± 0.04	1.15 ± 0.09	0.96 ± 0.10	0.80 ± 0.08	0.04*
Phosphate (mmol/day) (10.90-32.30)	Free-choice	30.3 ± 1.0	30.1 ± 1.9	30.2 ± 1.7	30.6 ± 1.9	0.98
	Low calcium	24.4 ± 1.0	24.9 ± 2.1	23.4 ± 1.4	25.3 ± 1.9	0.72
	P (Fc versus Lc)	<0.001	<0.001	<0.001	<0.001	
Magnesium (mmol/day) (<12.0)	Free-choice	4.54 ± 0.16	4.63 ± 0.27	4.38 ± 0.24	4.65 ± 0.31	0.72
	Low-calcium	3.90 ± 0.15	3.87 ± 0.28	3.90 ± 0.22	3.92 ± 0.28	0.99
	P (Fc versus Lc)	0.003	0.001	0.02	0.006	
Uric acid (μmol/day) (<4760)	Free-choice	3191 ± 99	3170 ± 157	3243 ± 162	3146 ± 204	0.92
	Low calcium	3146 ± 111	3202 ± 206	3181 ± 184	3042 ± 192	0.83
	P (Fc versus Lc)	0.32	0.71	0.39	0.17	

The numbers in parentheses indicate the normal range of values.

\*Post hoc analysis  $P < 0.05$ , group 1 versus group 3.



**Fig. 3.** Differential effect of a low-calcium diet on PTH and 1.25 vitamin D according to bone mineral density at lumbar spine. Groups are indicated by numbers: 1 = group 1, z-score at lumbar spine <-0.5; 2 = group 2, z-score at lumbar spine -0.5-0.5; 3 = group 3, z-score at lumbar spine >0.5. ANOVA  $P = 0.03$  for the 1.25 vitamin D-to-PTH ratios under low-calcium diet, post hoc analysis  $P < 0.05$  for groups 1 versus 3. Error bars indicate SEM.



**Fig. 4.** Hypercalciuric patients and bone mineral density at lumbar spine. Group 1: z-score <-0.5,  $n = 30$ ; group 2: z-score -0.5-0.5,  $n = 36$ ; group 3: z-score >0.5,  $n = 28$ . Numbers in the bars indicate the number of normocalciuric (blank areas) or hypercalciuric (hatched areas) patients in the respective groups.

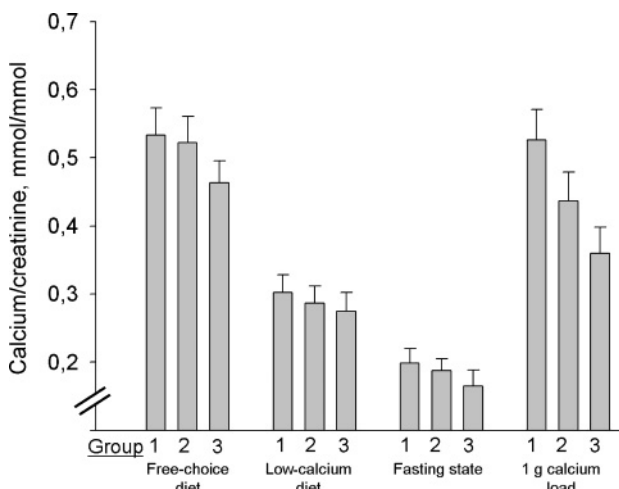
There was a significant association among low BMD and the percentage of hypercalciuric patients (Figure 4) with 47% hypercalciuric patients in group 1, 22% in group 2 and 18% in group 3 (chi-square  $P = 0.03$ ). An analysis of the mineral metabolism in the male participants of the study yielded results comparable with those of the entire study population (results not shown). The z-score at the lumbar spine of all hypercalciuric patients ( $n = 27$ ) was  $-0.34 \pm 0.15$ , and the corresponding value of all normocalciuric patients ( $n = 67$ )  $0.20 \pm 0.12$  ( $P = 0.01$ ) (Table 4). Similarly, the z-scores at the femoral neck and the tibial dia- and epiphysis were higher in normo- than in the hypercalciuric patients (Table 4).

Figure 5 shows calciuria, expressed as calciuria/creatinine in mmol/mmol for direct comparison of 24-h, 2-h and 4-h urines, while on the different diets. Calciuria declined from group 1- to group 3 on all four dietary conditions (ANOVA  $P = 0.009$ , post hoc analysis significant for group 1 versus 3 at  $P < 0.01$ ), an effect numerically most pronounced after the 1 g calcium load. When all patients were considered and grouped into normo- and hypercalciuric patients, the switch from a free-choice diet to a low-calcium diet caused similar changes of PTH and 1.25 vitamin D in patients with low BMD (Figure 6A), whereas a differential effect was observed for patients with a normal or high BMD (Figure 6B).

**Table 4.** DEXA z-scores of hypercalciuric and normocalciuric patients

Patients	Lumbar spine	Femoral neck	Tibial diaphysis	Tibial epiphysis
Hypercalciuric, <i>n</i> = 27	-0.34 ± 0.15	0.08 ± 0.13	0.34 ± 0.26	-0.64 ± 0.20
Normocalciuric, <i>n</i> = 67	0.20 ± 0.12	0.36 ± 0.13	0.54 ± 0.12	-0.15 ± 0.11
<i>P</i> *	0.01	0.21	0.44	0.03

\**P*-value of hypercalciuric versus normocalciuric patients (*t*-test for unpaired observations).



**Fig. 5.** Impact of different regimens on urinary calcium excretion in subjects grouped according to bone mineral density at lumbar spine. Calcium excretion and creatinine were measured in 24-h urines under free-choice diet and low-calcium diet, in 2-h urines after 12-h fasting, and in 4-h urines after a 1-g calcium load. Data are expressed as calcium/creatinine, mmol/mmol. Group 1: z-score < -0.5, *n* = 30; group 2: z-score -0.5–0.5, *n* = 36; group 3: z-score > 0.5, *n* = 28. Error bars indicate SEM.

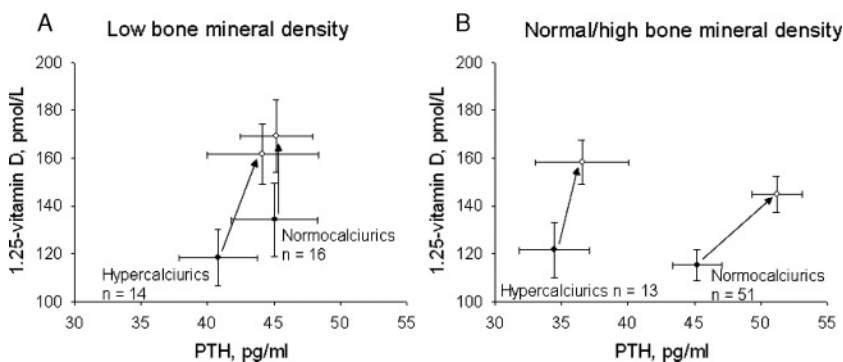
## Discussion

In the present study a cohort of 94 unselected renal calcium stone formers was investigated on a free-choice diet, followed by a low-calcium diet for 1 week and a 1 g oral calcium load test after 12 h of fasting (Figure 1). When

all patients were considered as one group, dietary calcium restriction caused a slight but significant decline in plasma calcium concentration associated with an increase in PTH and 1.25 vitamin D levels and a substantial reduction of calciuria (Figure 2). Thus, the anticipated physiologic response to the low-calcium diet was observed, suggesting a high degree of compliance of our patients to the diet.

In previous studies investigating the relationship between calcium stone formers and reduced BMD, patients were classified according to the amount of urinary calcium excretion, followed by an analysis of the corresponding BMD [2–5,17]. The focus of our investigation was different. We first grouped the calcium stone formers into three tertiles according to their lumbar BMD and then analysed the mineral metabolism on free-choice and low calcium diet as a function of BMD. To avoid a bias caused by the distribution of z-scores, which represent a standard deviation score, patients were grouped into cohorts representing one standard deviation each (z-score group 1: < -0.5, group 2: -0.5–0.5 and group 3: > 0.5). Interestingly, when the patients were grouped according to their BMD, a differential BMD-dependent response of PTH and 1.25 vitamin D to the low calcium diet was observed.

Urinary calcium excretion increased with declining BMD (Figure 5). This phenomenon is not explained by a differential nutritional behaviour during the study periods, because it was also present while fasting and after the standardized 1-g calcium load. The percentage of subjects with hypercalciuria (fasting or absorptive) according to the definition provided on the basis of previous studies [2,4,8,12,14] declined in our study from 47% in patients with the lowest mineral bone density to 18% in patients with the highest density. This finding is in line with the reported predictive value of calciuria on bone loss in stone formers [18]. In line with previous reports [2–4,7,8], BMD was diminished in hypercalciuric when compared to normocalciuric patients (Table 4). For most hypercalciuric patients reported in the literature so far, the underlying hypercalciuria-causing mechanism is unknown and the search for monogenic diseases was mostly unsuccessful [19]. One reason for the absence of success might be the difficulty to define unambiguous phenotypes. In this regard, challenging dietary conditions, as the ones used in



**Fig. 6.** Mineral metabolism in hypercalciuric and normocalciuric patients with low (A) and normal/high (B) bone mineral density. Arrows indicate the changes in mineral metabolism when patients were switched from free-choice to low-calcium diet. Low bone mineral density: DEXA z-score at lumbar spine < -0.5; normal/high bone mineral density: DEXA z-score at lumbar spine > -0.5. Error bars indicate SEM.

the present study, might ultimately help to define discrete entities. For clinical practice, the observation that hypercalciuric patients are at risk to lose bone mass is relevant and supports the treatment of hypercalciuric stone formers with a thiazide diuretic. This group of diuretics enhances renal calcium reabsorption, preserves BMD [20,21] and reduces the risk of bone fractures [22].

The finding of a link between BMD in calcium stone formers and the responses of 1.25 vitamin D and PTH to a low-calcium diet has not been described before (Figure 3). Starting from a homogenous baseline on free-choice diet (Figure 3), 1.25 vitamin D and PTH levels differentially increased on the low-calcium diet: in group 1, the group of patients with the lowest z-scores, the increase of 1.25 vitamin D was most pronounced, whereas for PTH the largest increase was found in group 3, the patients with the highest z-scores (Figure 3). The putative mechanisms explaining the BMD-related differential regulation of mineral metabolism (Figure 3) were not addressed by this study and are still speculative. Some of them are discussed hereafter.

- (a) High phosphate concentrations stimulate PTH release [23] and inhibit the  $1\alpha$ -hydroxylase [24], thus regulating PTH and 1.25 vitamin D concentrations. Since both plasma and urinary phosphate concentrations were of the same magnitude in the three groups, phosphate is unlikely to explain the BMD-dependent apparent low-calcium diet induced regulation of PTH and  $1\alpha$ -hydroxylase.
- (b) Hypocalcaemia stimulates both the excretion of PTH [25] and the activity of the  $1\alpha$ -hydroxylase [26]. However, the small changes observed in calcium concentrations are similar in all three groups and therefore might not account for a differential regulation. Nevertheless it is conceivable that the concentration response curve between calcium and PTH or calcium and  $1\alpha$ -hydroxylase might be different in the three groups.
- (c) Since PTH is a powerful stimulus of the  $1\alpha$ -hydroxylase [27,28], one might interpret the present finding as a PTH resistance in group 3 or a hypersensitivity in group 1. The  $1\alpha$ -hydroxylase response to PTH is blunted in acidosis [29]. However, the absence of significant differences of plasma pH, plasma bicarbonate and urinary citrate levels between the three groups argues against this possibility. Furthermore, given the same amount of urinary excretion of urea and sulfate in all three groups of patients investigated, it is unlikely that different amounts of proteins, the main nutritional source of protons, were consumed.
- (d) The renal  $1\alpha$ -hydroxylase decreases whereas PTH levels increase with decreasing renal function [30]. The non-significant differences of plasma creatinine and creatinine clearance are however unlikely to explain this differential response.
- (e) The biologically active free serum concentration of 1.25 vitamin D is very low as 1.25 vitamin D is >99% bound to vitamin D binding protein and albumin [31]. In the present study as in all other investigations in the field, only the total concentrations of this steroid were assessed. Thus the observed differ-

ences might not be biologically relevant, a contention unlikely to be true because severe dysproteinemias have to be present for changing protein binding of vitamin D [32]. In conclusion, none of the five mechanisms mentioned is likely to account for the differential changes of PTH and 1.25 vitamin D after a low calcium diet.

Although the observation of a differential regulation of PTH and 1.25 vitamin D on a low calcium diet currently remains unexplained, the resulting PTH and 1.25 vitamin D levels at the end of the low calcium diet might explain the results from the 1 g calcium load. In the group with the highest 1.25 vitamin D levels (group 1), urinary calcium excretion was highest, and in the group with the lowest 1.25 vitamin D levels (group 3), calcium excretion was the lowest, suggesting an intact intestinal response for 1.25 vitamin D. The higher PTH concentrations in group 3, compared to group 1, might in addition favour renal calcium retention. The observation of relatively high 1.25 vitamin D concentrations in the absence of concomitantly increased PTH levels in group 1 with a low BMD is reminiscent of adynamic bone disease as it is encountered in patients with renal failure. These patients are characterized by a very low capacity to buffer calcium and to handle an extra calcium load [33]. Thus it is reasonable to speculate that our patients with low BMD following time periods with low-calcium intake exhibit normal or even exaggerated intestinal calcium absorption, but cannot deposit this extra amount of calcium into their bones. As a corollary, calciuria increases. If this reasoning was correct, then the primary defect causing the association of low BMD and renal calcium stone formation might be attributable to a primary bone problem, with a diminished capacity to utilize the absorbed calcium.

In conclusion, our study on calcium stone formers provides an observation on how they adapt to a low-calcium diet and shows a link between lumbar spine BMD and calciuria. Whether this association is a peculiar feature of stone-forming patients or also applies to non-stone formers cannot be answered by our study at the present time because of the absence of a cohort of non-stone formers analysed with the same protocol.

The observed differential response of PTH and 1.25 vitamin D after a 1-week challenge by a low calcium diet between calcium stone formers with high and low BMD appears teleologically to be sound: the primary regulator of intestinal calcium absorption, 1.25 vitamin D, is increased in patients with low BMD, whereas the principal activator of osteoclasts, PTH, is increased in patients with high BMD. These findings deserve further investigations in order to elucidate the mechanisms by which they appear, but are of high relevance by underscoring the relationship between low mineral bone density and hypercalciuria.

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